is demonstrable in animals treated with cortisone or thorotrast(1,4).

It is essential that toxin be given after the injection of the colloidal materials. When the order is reversed and toxin injected beforehand, no lesions of the skin or kidneys occur.

The observations add support to the previously stated hypothesis(1,3,4) that the reticuloendothelial system is in some way concerned with defense against the necrotizing effects of gram-negative bacterial toxins, and interference with the normal functioning of this system results in a new kind of vulnerability to toxin. The possibility that a comparable interference with detoxification may be the function of the first or "preparing" injection of toxin, in the local and generalized Shwartzman reactions, is a subject for further investigation.

*Summary.* The intravenous injection of colloidal carbon or saccharate of iron oxide produces, in the rabbit, an alteration in the reactivity to gram negative bacterial toxins which resembles the effect of cortisone, thorotrast and trypan blue. An intradermal injection of toxin in such animals causes a local reaction of hemorrhagic necrosis which is similar to the local Schwartzman reaction, and an intravenous injection causes the development of bilateral cortical necrosis of the kidneys resembling the generalized Shwartzman reaction. It is suggested that the colloidal materials may produce these effects by interfering with normal protective functions of the reticuloendothelial system.

1. Thomas, L., and Mogabgab, W. J., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 829.

2. Thomas, L., and Good, R. A., *J. Exp. Med.*, 1952, v96, 605.

3. Good, R. A., Denny, F. W., Jr., Thomas, L., *J. Clin. Invest.*, June 1952, v31, 632. (abstract)

4. Good, R. A., and Thomas, L., *J. Exp. Med.*, 1952, v96, 625.

5. Bennett, I. L., Jr., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 248.

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## Effects of d-Amphetamine on the Electroencephalogram of the Dog.* (20227)

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The central stimulating properties of sympathomimetic amines have been studied by observing the effects of these drugs on the spontaneous activity of rats. Schulte *et al.* (5) found that d-amphetamine produced the greatest increase in activity of 75 compounds studied. We felt that it might be of value to supplement such experiments on rats by observations on the electroencephalogram (EEG) of the dog. Although we have found no published reports on the EEG effects of d-amphetamine, studies have been made on the racemic form, *dl*-amphetamine. Gibbs and Maltby(2) found that this drug increased the frequency of the human EEG. In the rabbit, this drug potentiates the spread of a response from a stimulated area of the cortex to distant areas (Misrahy and Toman)(3).

The present paper describes an effect of *d*-amphetamine on the EEG of the dog. In the experiments described in Part I, dogs were prepared under thiopental anesthesia and then immobilized with decamethonium ($C_{10}$). This procedure permitted accurate recording of the EEG. To correlate the EEG changes produced by this drug with its effect on behavior, we used trained unanesthetized dogs, as described in Part II.

*Part I—Thiopental—$C_{10}$ Dogs. Procedure.* The method is based on that described by Schallek and Smith(4). Dogs were prepared under thiopental anesthesia. Artificial respiration was instituted through a tracheal cannula, while hypodermic needles were inserted

* A brief report on this work was presented at the Chicago meeting of the Am. Physiol. Soc., April 9, 1953. Abstract appeared in *Fed. Proc.*, 1953, v12, 126.

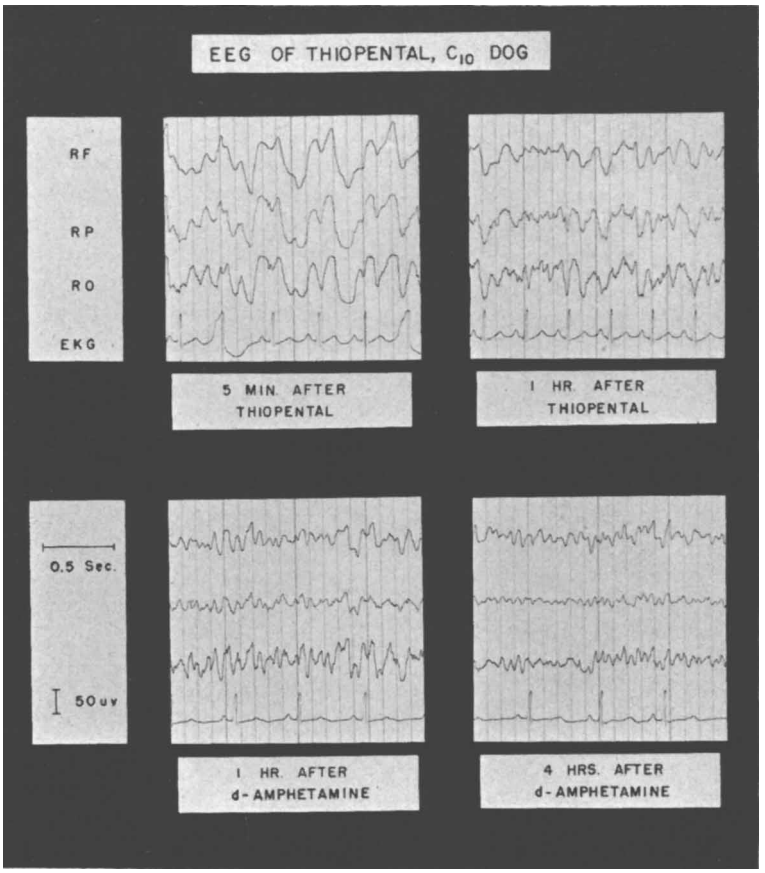


FIG. 1. EEG of thiopental-$C_{10}$ dog. First 3 lines in each record are the EEG, right frontal, right parietal and right occipital; the fourth line is the EKG, Lead II. Paper speed, 6 cm/sec. Aug. 27, 1952—Dog No. 229, ♀—6.5 kg; Record A—5 min. after thiopental, 10 mg/kg, I.V.; Record B—55 min. after A; *d*-Amphetamine, 10 mg/kg, I.V., inj. immediately after B; Record C—one hr after B; Record D—4 hr after B.

in a femoral vein for injecting solutions and in the opposite femoral artery for recording blood pressure. The EEG was recorded from steel needles inserted in holes drilled through the skull; a Grass Model III C electroencephalograph was used. The electrocardiogram (Lead II) was recorded on the same machine. On completion of the operative procedure, the areas around the incisions were infiltrated with procaine and the animals immobilized with decamethonium. The drug being tested was injected one hour after the last injection of thiopental. Records were taken at periodic intervals for four or five hours. At the conclusion of the experiment, the animal was sacrificed. For analysis of the results, the dominant frequency and amplitude were measured on typical sections of the records.

*Results.* Preliminary experiments indicated that the effects of *d*-amphetamine are maximal at 5 and 10 mg/kg, I.V. The EEG changes consist of a progressive increase in frequency combined with a decrease in amplitude (Fig. 1).

Ten additional experiments performed with *d*-amphetamine and ten control experiments permitted further analysis of the results (Table I). The control animals were injected with 10 ml 0.9% NaCl. The progressive increase in the frequency of their EEG's seems to be correlated with recovery from the thiopental. Although the animals were stirring half an hour after injection of this anesthetic, the EEG suggests that traces of the agent remained long after injection. Brodie *et al.*(1) found that appreciable amounts of thiopental

TABLE I. Effects of d-Amphetamine on EEG of Thiopental-$C_{10}$ Dogs.

| | Multiples of initial EEG frequency (each figure is avg of 10 animals) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Time after injection | | | | | | |
| | 0 min. | 15 min. | 30 min. | 1 hr | 2 hr | 3 hr | 4 hr |
| Controls | 1.00 | 1.03 | 1.09 | 1.19 | 1.59 | 1.69 | 2.03 |
| d-Amphetamine, 10 mg/kg, I.V. | 1.00 | 1.38 | 1.80* | 2.18* | 2 49 | 2.67 | 3.11 |
| Ratio: d-Amphetamine/controls | 1.00 | 1.34 | 1.65 | 1.83 | 1.56 | 1.58 | 1.53 |

* Denotes statistically significant difference when compared with control value ($P = .05$).

may be recovered from the dog twenty-four hours after administration.

The ratio of the *d*-amphetamine series to the controls indicates that the effects of this drug are maximal within the first hour after injection. This result accords with the observation in Part II that in unanesthetized dogs the peak action of *d*-amphetamine occurs within the first hour.

The question remains as to the nature of the EEG change produced by *d*-amphetamine. Is it merely some unimportant side-effect, or is it related to the central stimulation that is sought with this drug? To answer this question, we studied the effects of *d*-amphetamine on trained unanesthetized dogs, as described in Part II.

*Part II—Unanesthetized Dogs. Methods.* Dogs were trained to lie quietly on the table. Electrodes were attached to the shaved scalp with bentonite paste and then secured with collodion, using the method described in the "Instruction Manual for the Grass Electroencephalograph." The electrodes, made by the Grass Instrument Company for clinical use, are cup-shaped discs of pure silver, about 8 mm in diameter. Records were made of the following 4 types of behavior: *"Sleeping"* —the dog lies on the table completely relaxed, eyes tightly shut, paying no attention to ordinary laboratory sound. Records were not made until the animal had been in this state for several minutes. *"Dozing"*—the eyelids are lightly shut; the dog can be aroused by slight noises. *"Resting"*—the dog lies quietly with open eyes. *"Alert"*—the animal has just been aroused by the sound of a whistle or by tapping on the table. The signal had to be carefully graded to attract the dog's attention without causing movement, as the resulting muscle artifacts would obscure the EEG.

Drugs were injected intravenously. To avoid damage to the veins, only one injection was given to a dog on any one day; an interval of several days was allowed between injections. EEG records made following drug injection were compared with controls made on the same day before the drug was injected. Although it was easy to observe the effects of stimulant drugs on the behavior of unanesthetized dogs, it was more difficult to study the corresponding EEG changes. This is because doses of a drug producing sufficient central stimulation to change the EEG were apt to produce so much gross movement that the record was obscured by muscle artifacts.

*Results. A. Untreated Dogs.* As shown in Fig. 2, the EEG of a dog in deep sleep is composed of low frequency, high amplitude waves. As the dog is first gently awakened and then fully alerted, there is a rise in frequency, accompanied by a fall in amplitude. Comparison with Fig. 1 indicates that these changes are similar to those following the injection of *d*-amphetamine in thiopental-$C_{10}$ dogs.

*B. Drug Experiments.* These experiments, carried out on 3 different dogs, may be summarized by the abridged protocols in Table II. It is evident that with *d*-amphetamine, in doses above 0.01 mg/kg, I.V., there is: (1) a progressive increase in motor activity. The maximum effect occurs within the first hour. (2) some evidence for a corresponding increase in the frequency of the EEG. This increase appears most regularly in the "resting" record.

*Discussion.* The dominant frequency of the EEG in unanesthetized dogs has been shown to increase during periods of sensory stimulation (Swank and Watson) (6). A rise in the frequency of the human EEG has been shown to follow the shift from "eyes closed" to "eyes open while reading"; a similar though smaller

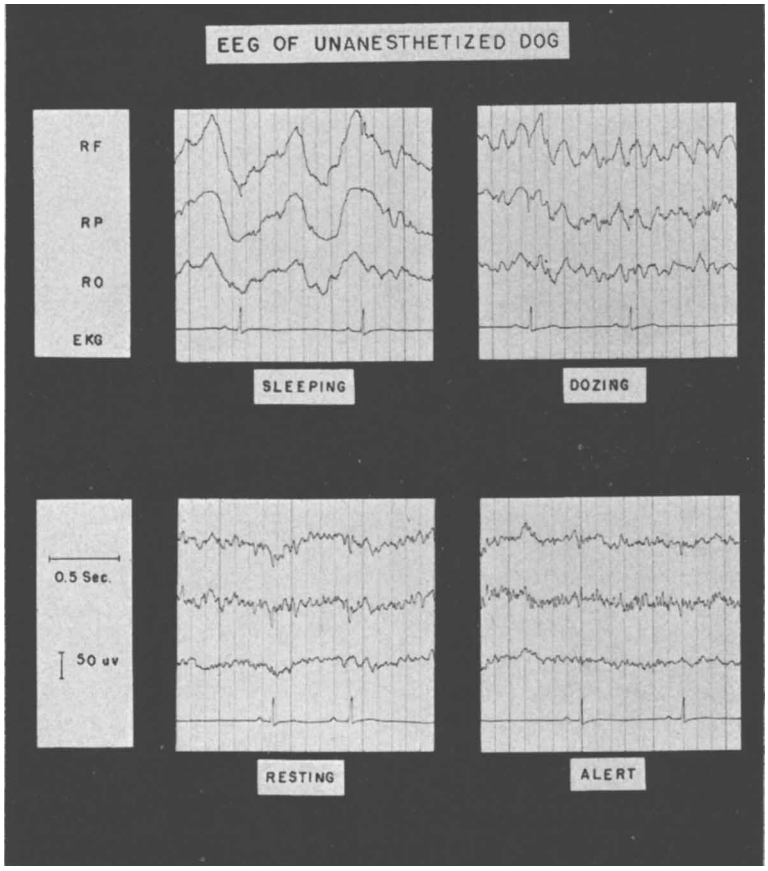


FIG. 2. EEG of unanesthetized dog. Arrangement as in Fig. 1. July 29, 1952—Dog No. 2, ♀—6.0 kg; Record A—sleeping; Record B—dozing; Record C—resting; Record D—alert.

shift follows the intravenous injection of 20 mg of *dl*-amphetamine (Gibbs and Maltby(2).

The present study on the dog agrees with this study on man in showing a parallel between the EEG effects of sensory stimulation and of *d*-amphetamine (compare Fig. 1 and 2). The evidence indicates that the effects of *d*-amphetamine on the EEG of the dog are parallel to its effects on behavior (Table II). The EEG changes introduced by *d*-amphetamine, therefore appear to be related to the central stimulation produced by this drug.

TABLE II. Effects of d-Amphetamine on Unanesthetized Dog.
Dog No. 1—♀, 6.3 kg.

| Date, 1952 | Dose of d-Amphetamine, mg/kg I.V. | Frequency of "resting" EEG Before inj. | After inj. | Change | Effects on behavior |
|---|---|---|---|---|---|
| 7/24 | .0002 | 28/sec. | 28/sec. | 0 | No change |
| 7/28 | .001 | 30 | 30 | 0 | " " |
| 7/31 | .010 | 30 | 32 | +2 | Some movement after inj. |
| 8/ 5 | .013 | 24 | 28 | +4 | Slight restlessness after 30 min. No desire for sleep |
| 7/18 | .015 | 26 | 34 | +8 | Had to be comforted and later restrained |
| 8/ 8 | .020 | 30 | 42 | +12 | After 30 min., restlessness & increased body movement |
| 7/17 | .030 | 30 | — | — | Too much movement to get any record |

*Summary.* (1) In dogs prepared under thiopental and then immobilized with $C_{10}$, the injection of *d*-amphetamine causes a progressive increase in the frequency of the EEG, with a corresponding decrease in amplitude. These effects are maximal within the first hour. (2) When unanesthetized dogs are gently roused from sleep and then fully alerted, there is a rise in the frequency of the EEG, accompanied by a decrease in amplitude. (3) When *d*-amphetamine is injected into unanesthetized dogs, there is an increase in motor activity, the peak effect being reached within the first hour. There is some evidence for an increase in the frequency of the EEG. (4) These findings suggest that the effects of *d*-amphetamine on the EEG of the dog are parallel to its effect on behavior.

1. Brodie, B. B., Bernstein, E.; and Mark, C. L., *J. Pharm. and Exp. Therap.*, 1952, v105, 421.

2. Gibbs, F. A., and Maltby, G. L., *J. Pharm. and Exp. Therap.*, 1943, v78, 1.

3. Misrahy, G., and Toman, J. E. P., *Fed. Proc.*, 1953, v12, 352.

4. Schallek, W., and Smith, T. H. F., *J. Pharm. and Exp. Therap.*, 1952, v104, 291.

5. Schulte, J. W., Reif, E. C., Bacher, J. A., Jr, Lawrence, W. S., and Tainter, M. L., *J. Pharm. and Exp. Therap.*, 1941, v71, 62.

6. Swank, R. L., and Watson, C. W., *J. Neurophysiol.*, 1949, v12, 137.

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## Hormonal Influences on Liver Lipid Partition, Carbohydrate and Electrolyte Metabolism in the Rat. (20228)

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Among recent evidence indicating there exists an association between potassium and carbohydrate metabolism are the effects of insulin, epinephrine, and the infusion of large quantities of glucose upon the potassium level of plasma or serum. It was reported from this laboratory that following epinephrine and insulin injection in intact rats the plasma potassium level was lowered; this effect on potassium was also found after epinephrine in adrenalectomized or adrenal-demedullated rats but not after insulin(1-3). It was also reported that insulin and epinephrine induced divergent changes in the potassium content of liver and skeletal muscle(4-5). Further elucidation of the mechanism of action of insulin and epinephrine on the potassium content of plasma and tissues was desirable. Since it is increasingly evident that lipid and carbohydrate metabolism are interdependent phenomena, it seemed important to determine the effects of certain induced endocrine conditions upon liver lipid partition. In contrast to the present knowledge of the effects of hormones and their regulatory roles in carbohydrate and protein metabolism, information concerning hormonal influences upon lipid metabolism is limited. Experimental and clinical observations indicate that the endocrine secretions of the pancreas, thyroid, and adrenal cortex affect serum lipid partition(6-10). Evidence has been reviewed(11) which indicates that adrenal cortical homones either directly or through effects upon carbohydrate and protein metabolism have a critical influence upon lipid metabolism. Levin and Farber(12) reported increased total liver fat in the mouse following certain types of stress, but not after others. These investigators concluded that the stress per se did not initiate the "mobilization" of fat to the liver since it was prevented if the increased caloric requirements were met by the administration of glucose. However, Brownell, Hartman and Liu(13) reported experiments in the dog which indicated that there was a distinct hormone secreted by the

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